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L. Eggeling · S. Oberle · H. Sahm

Improved L-lysine yield with *Corynebacterium glutamicum*: use of *dapA* resulting in increased flux combined with growth limitation

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Abstract The amino acid L-lysine is produced on a large scale using mutants of *Corynebacterium glutamicum*. However, as yet recombinant DNA techniques have not succeed in improving strains selected for decades by classic mutagenesis for high productivity. We here report that seven biosynthetic enzymes were assayed and oversynthesis of the dihydrodipicolinate synthase resulted in an increase of lysine accumulation from 220 mM to 270 mM. The synthase, encoded by *dapA*, is located at the branch point of metabolite distribution to either lysine or threonine and competes with homoserine dehydrogenase for the common substrate aspartate semialdehyde. When graded *dapA* expression was used, as well as quantification of enzyme activities, intracellular metabolite concentrations and flux rates, a global response of the carbon metabolism to the synthase activity became apparent: the increased flux towards lysine was accompanied by a decreased flux towards threonine. This resulted in a decreased growth rate, but increased intracellular levels of pyruvate-derived valine and alanine. Therefore, modulating the flux at the branch point results in an intrinsically introduced growth limitation with increased intracellular precursor supply for lysine synthesis. This does not only achieve an increase in lysine yield but this example of an intracellularly introduced growth limitation is proposed as a new general means of increasing flux for industrial metabolite overproduction.

Introduction

The amino acids represent the major fraction of primary metabolites produced by microbes. They are used as food and feed additives. With *Corynebacterium glutamicum* currently about 700 000 tonnes of L-glutamate and 300 000 tonnes of L-lysine are produced annually (Hodgson 1994; Leuchtenberger 1996). This production occurs with classically obtained mutants, which have been derived by many rounds of undirected mutagenesis and screening for increased productivity. This classic procedure to derive producer strains is complemented by molecular techniques which, in principle, are directed to tailoring the carbon flux by overcoming limitations, reducing competing biosynthetic pathways or reducing degrading activities. An example of such molecular strain construction is the improvement of L-tryptophan-producing *Escherichia coli* (Berry 1996; Dell and Frost 1993) or the development of L-isoleucine-producing strains of *C. glutamicum* (Morbach et al. 1995, 1996a, 1996b).

However, with high productivity it is becoming increasingly difficult to raise the performance of strains by pathway tailoring. In fact, improvement by molecular techniques is as yet unknown for any strain producing high levels of L-glutamate or L-lysine, although molecular studies have supplied profound new insights into lysine synthesis with *C. glutamicum* (Eggeling 1994; Eggeling et al. 1996). This relates to the unique split pathway by which lysine is synthesized (Schrumpf et al. 1991; Sonntag et al. 1993) and the identification of three flux-controlling steps. Control is exerted at the entry of aspartate into the assembling pathway (Kalinowski et al. 1991) and also at the branching point where aspartate semialdehyde is converted to either lysine or threonine (Cremer et al. 1991). Most exciting is the discovery that additional control is exerted by the export of L-lysine from the cell (Schrumpf et al. 1992; Bröer et al. 1993), and that a new type of carrier protein is present in *C. glutamicum* which serves as a valve to regulate the intracellular lysine concentration (Vrljic et al. 1996).

As part of our systematic analysis of lysine synthesis in *C. glutamicum*, we here present the analysis of whether the overexpression of individual genes of biosynthetic enzymes can improve a classically derived high-level lysine producer. In fact, one enzyme activity

L. Eggeling (✉) · S. Oberle · H. Sahm
Institut für Biotechnologie, Forschungszentrum Jülich GmbH, D-52425 Jülich, Germany
Tel.: + 49 2461 61 5132
Fax: + 49 2461 61 2710
e-mail: l.eggeling@fz-juelich.de

was found to affect the lysine yield markedly. The detailed flux studies revealed that, instead of simply tailoring the pathway by removing a bottleneck, the increased enzyme activity has far-reaching consequences with favourable global effects on the carbon flux within the aspartate family of amino acids and even within the central metabolism.

Materials and methods

Strains and growth

The parent *Corynebacterium glutamicum* strains used for this work were the wild-type ATCC13032, the low-level L-lysine producer DG52-5, and the high-level producer MH20-22B (Schrumpf et al. 1992). For high L-lysine accumulation with MH20-22B the $CaCO_3$-containing minimal medium CGX was modified to contain 40 g/l ammonium sulphate, 100 g/l glucose and 300 mg/l L-leucine. To derive pool concentrations and growth rates, the strains were grown on minimal medium CGXII (Keilhauer et al. 1993).

Genetic engineering

In order to construct a strain with a second copy of *dapA* integrated into the chromosome, the 1.4-kb *Sph*I/*Bam*HI *dapA*-containing fragment of pJC20 (Cremer et al. 1990) was ligated with the mobilizable but non-replicative vector pEM1 (Schrumpf et al. 1991). The resulting plasmid pEM1::*dapA* was introduced via intergeneric conjugation (Schäfer et al. 1990) with *E. coli* into *C. glutamicum*. Kanamycin-resistant transconjugants were selected for stable integration of the vector into the chromosome. The same 1.4-kb *Sph*I/*Bam*HI fragment, but with the *Sph*I site blunted, was also ligated with the low-copy-number vector pKW3 (Kronemeyer et al. 1995). This had been *Eco*RI-cleaved, blunted and *Bam*HI-cleaved, resulting in pKW3::*dapA* after ligation. This plasmid, as well as the multy copy plasmid pJC23 containing *dapA* (Cremer et al. 1990), was introduced by electroporation into *C. glutamicum*.

Enzyme determinations

The dihydrodipicolinate synthase activity was quantified in a very sensitive and reliable assay using dihydrodipicolinate reductase as the coupling enzyme. The assay contained, in a final volume of 1 ml at 30 °C, 4.4 µmol aspartate semialdehyde, 0.1 mmol TRIS pH 7.5, 1 µmol L-threonine, 2.5–10 µl crude extract (about 10 mg protein/ml), 0.3 µmol NADPH, and 10 µl heated extract prepared from *E. coli* RDA8pDB17. This latter heated extract (crude extract incubated for 3 min at 70 °C) of the *E. coli dapA* mutant overexpressing plasmid-encoded *dapB* (Richaud et al. 1986) served as a source of the dihydrodipicolinate reductase. The synthase activity reaction was started by adding 10 µmol sodium pyruvate. The homoserine dehydrogenase activity was measured as described (Reinscheid et al. 1994).

Analytical procedures

Growth was followed by absorbance measurements, and rates derived from the regression of at least seven data points. Amino acids were quantified by automatic precolumn derivatization with *o*-phthaldialdehyde, separated by reversed-phase chromatography and detected fluorometrically (excitation 230 nm, emission 450 nm) using an HP1090 Chem station (Hewlett-Packard). To derive pool concentrations, samples were directly taken from growing cultures and immediately processed by the silicone oil centrifugation method to produce a cytosolic fraction of metabolites (Klingenberg and Pfaff 1977). The required cytosolic volume was determined according to Rottenberg's method using [^{14}C] taurine as a non-penetrating marker and ^3H_2O as the water-permeable marker (Rottenberg 1979).

Results

Individual gene expressions and lysine production

The lysine pathway, the relevant genes and the enzymes they code for are given in Fig. 1. The plasmid-encoded genes were introduced into the excellent lysine producer *C. glutamicum* MH20-22B, obtained by classical

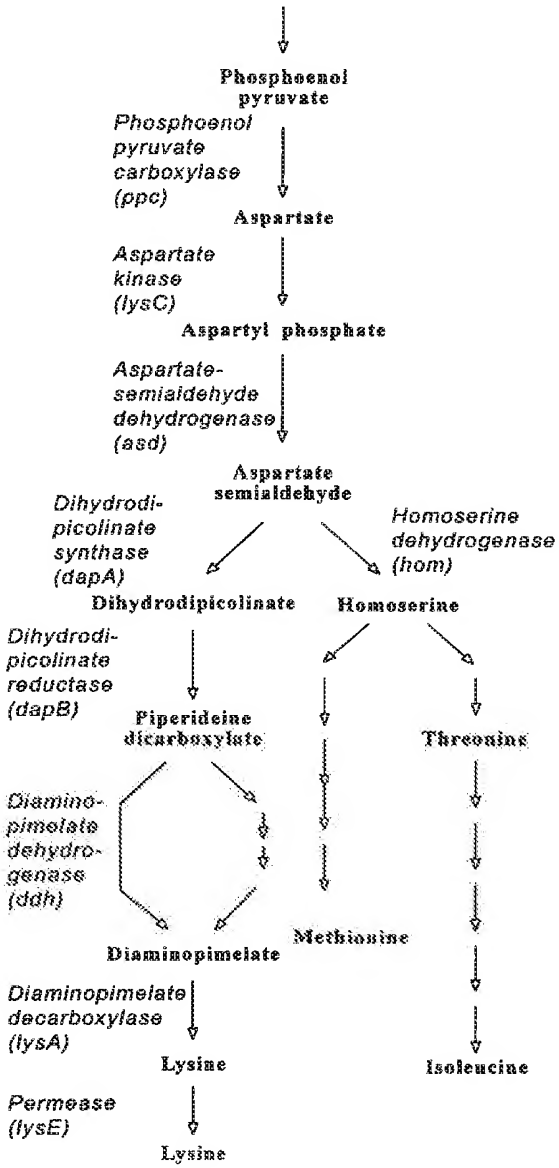

Fig. 1 Lysine biosynthesis as integrated within the aspartate family of amino acids. The relevant enzymes are given, as well as the genes used in this study and selected key intermediates

procedures (Schrumpf et al. 1992), which has been shown to possess feedback-resistant aspartate kinase (Kalinowski et al. 1991) and high lysine export activity (Bröer et al. 1993). It was confirmed by enzyme activity determinations that the plasmid-encoded genes were overexpressed in the respective strain more than sixfold. This is shown in Table 1, where, for comparison, the enzyme activities of the plasmid-less strain are given in parentheses. The nine recombinant strains, together with a control, were cultivated repeatedly (up to ten times) to determine the lysine accumulating. Most of the genes did not affect the yield and *ddh* even reduced it. However, *dapA* over expression has a pronounced effect on lysine accumulation. For comparison, the data for the low-level producer DG 52-5 and the wild type are included in Table 1, showing that, in these strains, *dapA* overexpression also results in increased lysine concentrations. This positive effect obtained by increased dihydrodipicolinate synthase activity would at first sight be interpreted as the simple removal of an existing "bottleneck".

Increased excretion rates and shortage of homoserine

The yield increase obtained is, of course, a major success from an industrial point of view. However, it is less informative for the understanding of cellular flux control where flux rates are required. In the few cases investigated, these flux rates are documented as varying considerably with process time (Morbach et al. 1996a; Vallino and Stephanopoulos 1993) We therefore assayed whether, under constant conditions of exponential growth, the *dapA* effect is operative. As can be seen from Table 2, this is the case with *C. glutamicum* MH20-22B where, owing to pJC23 carrying *dapA*, the lysine excretion rate was increased from 6.3 to 9.6 nmol min^{-1} mg dry $weight^{-1}$. Fortunately an increase in the excretion rate from 0 to 2.9 nmol min^{-1} mg dry $weight^{-1}$ was detectable also with the wild type of *C. glutamicum* (Table 2), thus preventing the problem of quantifying relatively small flux increases above large basal levels of fluxes. It is important to note that, as a second consequence of *dapA* overexpression, the detailed rate determinations also revealed a drastic growth rate reduction in both strains analysed because of increased *dapA* expression.

Following this surprising observation we considered whether the reduced growth rate could be due to the limiting availability of some amino acids. Unexpectedly, the addition of homoserine restored the growth rate to some extent (Table 2) but entirely abolished lysine excretion in the wild type. These effects of growth rate increase and lysine flux decrease were also present with

Table 1 The effect of increased enzyme activities, as obtained by individual gene overexpression, on L-lysine accumulation with *Corynebacterium glutamicum* MH20-22B. For comparison, the data obtained with the same plasmids for the low-level producer DG 52-5 and the wild type are also included. The first line shows the L-lysine accumulation by non-recombinant strains, without genes overexpressed (control). *n* the number of independent experiments. Increased lysin concentrations are given in bold face. *DH* dehydrogenase, *DDP* dihydrodipicolinate, *DAP* diaminopimelate, *PEP* phospho*enol*pyruvate

Oversynthesized enzyme	Overexpressed gene	Specific enzyme activity (μmol min^{-1} mg^{-1})		Lysine (mM) in *C. glutamicum* strain		
				MH20-22B	DG 52-5	Wild type
–	–			220 ± 10 (n = 7)	43	0
PEP carboxylase	*ppc*	1.45	[0.16][a]	230	**49**	0
Aspartate kinase	*lys*CS381F	0.11[b]	[0.01]	220 ± 13 (n = 6)	**48**	**38**
Aspartate semialdehyde DH	*asd*	0.12	[0.02]	230	38	0
DDP synthase	*dapA*	0.72	[0.06]	**272 ± 22** (n = 6)	**48**	**11**
DDP reductase	*dapB*	1.24	[0.05]	225	41	0
DAP DH	*ddh*	1.64	[0.18]	157 ± 8 (n = 6)	39	0
DAP decarboxylase	*lysA*	0.13	[0.02]	225	40	0
Aspartate kinase + DDP synthase	*lys*CS381F + dapA	0.13[b] + 1.28		**277 ± 10** (n = 10)	**68**	**45**

[a] In the square brackets the specific activity for the respective enzyme in the non-recombinant ancestor strain MH20-22B are given
[b] The aspartate kinase used, encoded by the *lys*CS381F gene, is not inhibited by 60 mM L-lysine plus 60 mM L-threonine

Table 2 Increased lysine excretion rates and shortage of homoserine in *C. glutamicum* strains upon overexpression of *dapA*

Strain	Supplementation (5 mM)	Lysine excretion (nmol min^{-1} mg dry $weight^{-1}$)	Growth rate (h^{-1})
MH20-22B	–	6.3	0.27
MH20-22B pJC23	–	9.6	0.19
	Hse	7.9	0.26
13032	–	0	0.37
13032 pJC23	–	2.9	0.12
	Hse	0	0.20
	Met, Thr	0	0.22
	Arg	2.0	0.13

L-methionine plus L-threonine addition and also with strain MH20-22B, but not with L-arginine or L-glutamate or L-serine (not shown). Since all the amino acids reversing the growth limitation are homoserine-derived, this clearly shows that the branch point of metabolite distribution via the synthase and homoserine dehydrogenase is involved in its entirety. This is surprising, since in vitro studies with homoserine dehydrogenase have shown that this enzyme is regulated by allosteric mechanisms in response to the L-threonine concentration (Miyajima et al. 1968). Therefore, one would expect that, at limiting L-homoserine and L-threonine concentrations, presumably present at the reduced growth rates (see below), these would counteract the drain-off of aspartate semialdehyde towards lysine. However, in vivo at high *dapA* expression this is apparently not the case.

Graded *dapA* expression

To verify the total synthase activity as the primary cause of the severe flux alterations within the aspartate family of amino acids, the effect of moderate *dapA* expression was studied. For this purpose, intergeneric conjugation between *E. coli* S17-1 and the wild type of *C. glutamicum* was performed to yield *C. glutamicum* ATCC13032::*dapA* with a second copy of *dapA* integrated into the chromosome. In addition, plasmid pKW3 was used, which is present in about 5 copies (Kronemeyer et al. 1995), thereby resulting in about 6 copies of *dapA*. Thus, in total, a series of *C. glutamicum* ATCC13032 derivatives with 1, 2, 6 or about 20 copies of *dapA* were generated (Table 3). The successful engineering of the strains was confirmed by Southern blots and plasmid analysis (not shown).

Enzymological analysis served to characterize the activities of the dihydodipicolinate synthase as well as the homoserine dehydrogenase as the other branch-point enzyme (Fig. 1). As can be seen in Table 3, the synthase activity in the wild type was increased in a graded fashion in the set of strains made. Strain AS80, also possessing two copies of *dapA*, was obtained from A. Schwarzer, University of Bielefeld, Germany. The different synthase activity of this strain and of strain 13032::*dapA* was repeatedly confirmed, and could be due to different fragments being used for strain construction. Significantly, the homoserine dehydrogenase activity, as the other reaction consuming aspartate semialdehyde, was not affected by increased *dapA* expression in any strain.

Since in *C. glutamicum* the homoserine dehydrogenase gene *hom* is repressed by methionine addition (Follettie et al. 1988; Miyajima and Shiio 1971), and this addition results in increased lysine flux (Vrljic et al. 1995), we used this effect as well to quantify the effects of aspartate semialdehyde distribution as influenced at the dehydrogenase branch. As can be seen from Table 3, in a culture supplied with methionine the dehydrogenase activity is reduced, but that of the synthase is unaffected. Therefore, by entirely different means, and by gentle genetic techniques, a modulation of the branch-point enzyme activities was obtained.

The effect of modified branch-point enzyme activities on fluxes and pools

The physiological consequences of the strains generated are included in Table 3. Both the reduced growth and increased lysine flux were already present with 2 *dapA* copies. This shows the exceptionally delicate flux balance at the branch point. Although the degree of flux increase and growth rate reduction is different for each strain, there is an overall correlation of increased synthase activity with decreased flux towards homoserine present within the series of strains constructed. The flux change achieved at the branch point by *hom* repression takes on a middle position.

For a verification of the flux changes, the intracellular situation within the strains made was analysed. For this purpose, cells taken from exponentially growing cultures were disrupted by the rapid silica-oil method, and the concentrations of amino acids in the intracellular pool were determined. As can be seen from Table 4, the intracellular concentrations of L-homoserine and L-threonine were reduced in response to the increased synthase activity, consistent with the fact that homoserine restored growth. On the other hand, the intracellular L-lysine concentrations were increased, thus causing the excretion by the specific lysine exporter (Vrljic et al.

Table 3 The effect of various *dapA* copy numbers on branch-point enzyme activities, and consequences for growth rates and excretion rates. The respective consequences of L-methionine addition are also included

Plasmid, vector or addition	*dapA* status (copy number)	Specific activity (μmol min^{-1} mg $protein^{-1}$)		Growth rate (h^{-1})	Excretion rate (nmol min^{-1} mg dry $weight^{-1}$)
		DDP synthase	Homoserine DH		
13032	1	0.051	0.62	0.43	0
13032::*dapA*	2	0.072	0.58	0.37	0.25
AS80	2	0.13	0.74	0.37	0.83
13032 + Met	1	0.047	0.16	0.37	0.92
13032 pKW3::*dapA*	6	0.25	0.62	0.36	2.7
13032 pJC23	20	0.63	0.67	0.22	3.8

Table 4 Intracellular amino acid concentrations in strains at the end of the exponential growth phase. *ND* not done

Strain/addition	Intracellular amino acid concentrations (mM)					
	Lys	Hse	Thr	Ala	Val	Asp
13032	3	5	9	11	3	21
13032::*dapA*	6	2	3	17	6	15
13032 + Met	12	≤1	ND	24	9	18
13032 pKW3	10	2	≤1	15	8	14
13032 pJC23	37	≤1	≤1	19	9	11

1995). Also in accord with the increased L-lysine flux, and with the theoretical understanding of flux control (Kacser and Burns 1981) is the reduced L-aspartate concentration, which is a consequence of increased flux through the aspartate kinase. Very surprising, however, is the considerable increase in the cytosolic L-valine and L-alanine concentrations. Both amino acids derive from pyruvate, without any need for further carbon components. On the basis of this result, it must be concluded that an increase of the intracellular pyruvate concentration is also an effect of synthase oversynthesis. It shows that the "*dapA* effect" even extends beyond effects within the aspartate family of amino acids.

Kinetic characterization of branch-point enzymes

The previous results show that the *dapA* effect on the cellular carbon fluxes must be due to the enzymological properties of both the synthase and the dehydrogenase. Consequently, the kinetic characteristics of these enzymes competing for the aspartate semialdehyde were determined in detail. It is known that the synthase is not regulated at the genetic level and not controlled in its catalytic activity (Cremer et al. 1988), whereas the dehydrogenase is controlled in its synthesis and by the intracellular threonine concentration to provide a regulatory feedback-control loop (Miyajima et al. 1968) (Fig. 2). For both the synthase and the dehydrogenase, initial reaction velocities were determined with aspartate semialdehyde as substrate. The steady-state kinetics of the enzymes is given in Fig. 3. To enable the comparison of the measurement data with metabolite fluxes in the living cell (see Discussion), the original data obtained in vitro with crude extracts were multiplied by 1.7 to convert them to specific activities based on dry weight (mg). From the original measurement data a V_{max} of 0.09 μmol min^{-1} mg $protein^{-1}$ for the synthase and of 0.75 μmol min^{-1} mg $protein^{-1}$ for the dehydrogenase was derived. The K_m values are 2.08 mM and 0.373 mM respectively, illustrating that the synthase has roughly a sevenfold lower maximal velocity and affinity. Figure 3 includes the kinetics of the synthase at twice the normal level (two copies of *dapA*). This analysis shows that the flux towards L-lysine is determined by the affinity of the synthase and depends on its total amount, indicating that not only the dehydrogenase is decisive for the flux distribution at the branch point. This forces one to

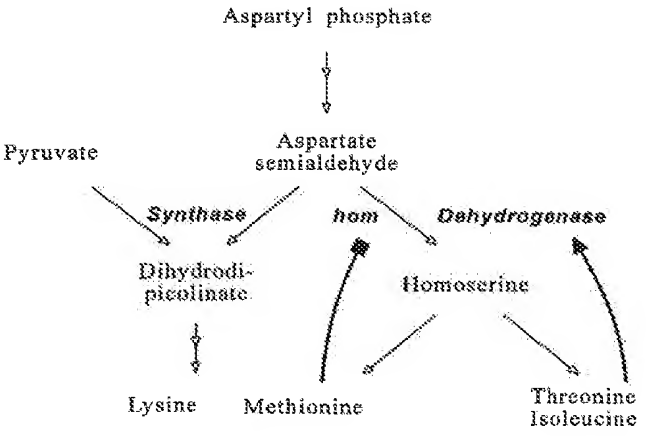

Fig. 2 The branch point of aspartate semialdehyde distribution with regulation of homoserine dehydrogenase by feedback control of enzyme activity (⟶) or regulation at the level of gene expression (⟶)

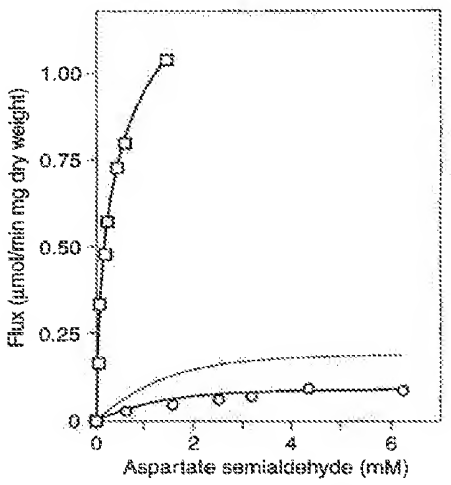

Fig. 3 In vitro steady-state kinetics of dihydrodipicolinate synthase (○) and homoserine dehydrogenase (□) as determined in extracts of the wild type of *Corynebacterium glutamicum* obtained from exponentially growing cells. The data have been multiplied by 1.7 to convert them, on the basis of the protein content, to specific activites based on dry weight. Also given is the synthase activity at the twofold elevated level (—). The maximal in vivo flux of the wild type through the homoserine dehydrogenase is only 0.00398 μmol min^{-1} mg dry $weight^{-1}$

consider the branch point as a whole, with both enzymes as one system designed to control the distribution of aspartate semialdehyde. Most importantly, even the regulatory features of the central metabolism are connected with the regulation of the branch-point enzymes, which alltogether results in a favourable intracellular flux situation to obtain a high lysine yield.

Discussion

As mentioned in the Introduction, very good amino acid producers have been achieved by classical breeding in the course of decades and it is extremely difficult to increase the yield in such producers by applying recombinant DNA techniques. This may be due to the fact that, with current recombinant DNA approaches, essentially "tailoring" of the pathways has been performed and only very recently have further metabolic reactions also been considered for strain development. This applies to the analysis of export genes of amino acids (Vrljic et al. 1996), for example, or to the quantification of reverse fluxes in the central metabolism (Marx et al. 1996).

As can be seen from the present work, specific *dapA* expression in *C. glutamicum* represents an ideal possibility of increasing the lysine yield, and we hypothesize that the related strains, *Brevibacterium lactofermentum* and *B. flavum*, also used for lysine production, can similarly be improved by increased synthase activity. The lysine flux can already be perceptably increased by gentle *dapA* expression. Owing to the quantified flux rates and pools, it is clear that simply considering the synthase as a bottleneck is not appropriate to describe the beneficial and in part dramatic effects of *dapA* expression. Instead, the kinetic properties of the synthase, together with the dehydrogenase, are decisive for the flux distribution and not, as previously assumed, the regulation of the dehydrogenase by feedback control (Miyajima et al. 1968) and repression of its structural gene (Folletie et al. 1988; Miyajima and Shiio 1971). Even more surprising is the fact that the increased flux via the synthase branch results in a decreased flux at the dehydrogenase branch, although the feedback loop is present with threonine as the heterotrophic effector, and the dehydrogenase as the flux-controlling device (Fig. 2). Therefore, in vivo this loop is not appropriate for the existing situation with two copies of *dapA* resulting in increased aspartate semialdehyde flux towards lysine. Thus the synthase serves as a barrier within lysine synthesis, and the flux through this low-affinity enzyme is proportional to its total amount.

For an assessment of the enzyme kinetics it is very informative to compare the in vitro data with the in vivo fluxes. The in vivo fluxes can be derived by simply summing up the amino acids at the ends of both branches by quantifying the rates at which they accumulate in their final destinations (cell material, cytosolic pool and external medium). For the wild type of *C. glutamicum* growing at $\mu = 0.43\ h^{-1}$, the rates for the amino acids involved (in nmol min^{-1} mg dry $weight^{-1}$) are 0.03 diaminopimelate, plus 1.15 lysine and 0.07 homoserine, 1.68 threonine, 1.06 methionine plus 1.17 isoleucine (Marx et al. 1996). This adds up to a flux of 1.18 through the synthase branch and 3.98 to the dehydrogenase branch. Both these in vivo velocities are far below what can be determined in vitro, see Fig. 3, where the smallest unit on the abscissa (the in vitro flux) is 250 nmol min^{-1} mg dry $weight^{-1}$. From this graph, it follows that the accessible in vitro data are far outside that of the in vivo fluxes. This is also true of the aspartate semialdehyde concentration, which can be estimated from the in vivo homoserine concentration (Table 4), the concentration of $NADPH + H^+$ and the equilibrium of the dehydrogenase reaction (Black and Wright 1955) to be about 0.05 mM. Moreover, the sensitivity of these relatively low in vivo fluxes becomes clear since, with two *dapA* copies, the flux through the synthase is increased only from 1.18 to 1.31 nmol min^{-1} mg dry $weight^{-1}$, and it is decreased through the dehydrogenase from 3.98 to 3.34 nmol min^{-1} mg dry $weight^{-1}$. This shows that, for the in vivo quantification and prediction of metabolite fluxes, additional techniques are required beyond in vitro enzyme studies. These are provided, for example, by NMR quantifications together with metabolite balancing (Eggeling et al. 1996; Marx et al. 1996).

The cytosolic amino acid concentrations present in *C. glutamicum* support the view that the introduction of growth limitation makes more pyruvate available. Since pyruvate is the second substrate of the synthase (Fig. 2), there is an additional interaction with the branch point. Other metabolites or cofactors are also expected to be increased in order to finally influence the flux at many locations of the lysine synthesis. This is in accord with flux theory, stating that control is distributed over the entire assembling sequence, as shown for tryptophan synthesis in yeast (Kacser and Burns 1981). In terms of global control of the metabolism in *C. glutamicum*, the intracellular amino acid concentrations show that the catabolic and anabolic processes are not well harmonized. This could be one reason why *C. glutamicum* is so well suited as an amino acid producer. In fact, one specific kinase activity [Hpr(Ser) kinase], shown to play a central role in *Bacillus subtilis* for the coordination of sugar uptake and catabolism, has not been detected in *C. glutamicum* (Saier et al. 1996).

In conclusion, the present study shows that even a classically obtained, very good amino-acid-producing strain can be improved. However, the improvement does not only consist in the removal of a bottleneck, but is due to a subtle flux redistribution at the dehydrogenase/synthase branch point. Combined with this redistribution is an introduced growth limitation, which results in increased availabilities of metabolites within the central metabolism. In fact, for years process engineering has been using extracellular constraints, like limited supply of ammonium or of any medium components to restrict growth, thereby extending the period of increased

product accumulation (Kiss and Stephanopoulos 1991; Konstantinov et al. 1991). This is suggested to be due to an increased availability of intracellular precursors. The present case of *dapA* demonstrates a relation of flux increase towards product with an intracellularly introduced growth limitation. Therefore, similar growth limitations, introduced by recombinant DNA techniques, are proposed as an attractive means for the improvement of further metabolite production processes.

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